AN AJSS An Asian Journal of Soil Science
Volume 11 | Issue 1 | June, 2016 | 207-212 | e ISSN–0976–7231 ■ Visit us : www.researchjournal.co.in

Research Article

DOI : 10.15740/HAS/AJSS/11.1/207-212

Co-inoculation of phosphate solubilizing bacteria and *Rhizobia* for improving growth and yield of mungbean (*Vigna radiata* L.)

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Received : 16.02.2016; **Revised :** 22.04.2016; **Accepted :** 18.05.2016

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Summary

Mung bean is an important pulse crop in West Bengal, growing nearly 11.7 thousands hectares of land. Scientific literature and case studies of inoculating of microbes by various scientists found significant response to crop growth. The present study was made to reduce fertilizer application rate by coinnoculating phosphate solubilizing bacteria and rhizobia for mung bean. The experiment was conducted in the year 2011 and 2012 at the 'Instructional Farm' Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal. Factorial Randomized Block Design was laid out with three replications and 10 treatment combinations. The treatments was without inoculation (A_0) and seed inoculation with *Rhizobium* and phosphate solubilizing bacteria (PSB) strain (A_1) as factor one and fertilizer treatments like untreated control (no fertilizer application B_0), application of NPK (recommended dose as basal B_1), 75 per cent recommended dose of N and P_2O_5 + 100% K_2O (B_2) and 50 per cent recommended dose of N and P_2O_5 + 100% K_2O (B_3). It was observed that inoculation with *Rhizobium* and phosphate solubilizing bacteria (PSB) along with 75 per cent RDF *i.e.* treatment combination (A_1B_2) was at par with treatment A_1B_1 *i.e.* 100 per cent RDF with respect to all the growth parameters and yield attributing characters of mung bean. So, it can be concluded that both 25 per cent nitrogenous and phosphatic fertilizer of the recommended dose can be substituted by seed co-inoculation with phosphate solubilizing bacteria and rhizobia without affecting the yield compared to 100 per cent RDF.

Key words : *Bacillus polymyxa*, Rhizobia, Mung bean, Seed inoculation

How to cite this article : Tarafder, H.K., Dey, A. and Dasgupta, S. (2016). Co-inoculation of phosphate solubilizing bacteria and *Rhizobia* for improving growth and yield of mungbean (*Vigna radiata* L.). *Asian J. Soil Sci.*, **11** (1) : 207-212 : **DOI : 10.15740/HAS/AJSS/11.1/207-212.**

Introduction

Govt. of India now focusing to increase area, production and productivity of pulse crop. Pulse growing area in India nearly 23 million ha with the annual production of 14.18 million tonnes and an average productivity of 617 kg ha^{-1} (GOI, 2009). Even being the largest producer in the World, our country has to import pulses about two million tones every year to feed our nation (Chaturvedi and Ali, 2002). Among the pulse crop mung bean [*Vigna radiata* (L.) Wilczek], commonly known as 'Moong or Mung bean'; is a good source of high quality protein (25%) with high digestibility (Payasi

el al., 2011). In West Bengal, about 4.4 thousand tonnes of mungbean production was recorded from 11.7 thousand hectares of area in 2005-06 (GOWB, 2006). It is well known that Mung bean being leguminous plant form symbiotic association by *Rhizobium* and thereby fixes atmospheric nitrogen (Singh *et al.*, 2011) and reduces nitrogen fertilizer requirement. Phosphorus is vital component of the substances that are building block of genes and chomosomes and the second major primary nutrient limiting plant growth is generally deficient in most of the soils due to its ready fixation (Schachtman *et al*., 1998). The increasing prices of phosphatic fertilizers have raised an alarming situation for our country. Therefore, it is very tricky for poor farmers to supplement P fertilizers in the soil to circumvent the P deficiency. The combined application of phosphate solubilizing bacteria and nodule forming bacteria in legumes stimulated plant growth (Algawadi and Gaur, 1988 and Perveen *et al*., 2002) as compared to their single inoculation.

Co-inoculation with N2-fixing and P solubilizing microbe was more effective approach for providing balanced plant nutrition (Gupta *et al*., 1998 and Martins *et al.*, 2004). Present study was designed to evaluate the co-inoculation potential of *Bacillus polymyxa* and *Rhizobium strain* (M-10) for improving growth, nodulation and yield of mung bean at different fertilizer levels.

Resource and Research Methods

The experiment was conducted in the month between March to May in the year 2011 and 2012 at the 'Instructional Farm' (22°95' north latitude, 88°50' East longitude), Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal. Soil was sandy-loam in nature, neutral in reaction pH 6.5, with available N (Subbiah and Asija, 1956), P (Olsen *et al*., 1954) and K (Brown and Wranckle,1988) was 178.7, 17.72 and 157.52.0 kg ha^{-1}, respectively. Factorial Randomized Block Design was laid out with three replications and 10 treatment combinations. The treatments was without inoculation (A_0) and seed inoculation with *Rhizobium* and phosphate solubilizing bacteria (PSB) strain (A_1) as factor one and fertilizer treatments like untreated control (no fertilizer application B_0), application of NPK (recommended dose as basal B_1), 75 per cent recommended dose of N and P_2O_5 + 100% K_2O (B_2) and 50 per cent recommended dose of N and P_2O_5 + 100% K_2O (B_3).

The *Rhizobium* strains (M-10) and phosphate solubilizing bacteria (*Bacillus polymyxa*) culture was collected from Nodule Research Laboratory, B.C.K.V. For A_1 treatment seeds were mixed thoroughly with the *Rhizobium* and phosphate solubilizing bacteria (*Bacillus polymyxa*) followed by shade drying for 30 minutes before sowing. In case of fertilizer treatment fertilizers were applied as basal. The green gram crop (cv. B-1) was sown at plant to plant spacing of 10 cm and row to row spacing of 30 cm apart. Other agronomic practices done as usual for pulse crop. Growth parameters like plant height, dry matter accumulation, crop growth rate, number and dry weight of nodules were recorded at different growth stages of crop and yield attributing characters and yield were noted at the time of harvest. The experiment was analyzed following the standard method of statistical analysis for factorial RBD.

Research Findings and Discussion

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Effect on growth attributes :

Inoculation with *Rhizobium* and phosphate solubilizing bacteria (*Bacillus polymyxa*) resulted in the higher rate of nitrogen fixation and phosphorus solubilization from insoluble phosphorus forms. From Table 1 it was revealed that there was significant difference in plant height due to application of *Rhizobium* and phosphate solubilizing bacteria (*Bacillus polymyxa*) seed inoculation. Highest and lowest plant height was 21.24 cm and 18.12 cm in the plots treated with innoculation (A_1) and without any culture (A_0), respectively at 30 DAS. The same trend was followed up to 60 DAS. The highest plant height of 23.96 cm and 52.55 cm at 30 and 60 DAS, respectively recorded in plot received the recommend dose of NPK followed by plot treated with 75 per cent recommended dose of N, P_2O_5 and 100 per cent K_2O.

Dry matter accumulations and crop growth rate (CGR) were observed. Inoculation with *Rhizobium* and phosphate solubilizing bacteria (*Bacillus polymyxa*) increased nodulation. As a result, dry matter accumulation was significantly higher (46.04 and 383.19 g m^{-2} at 30 and 60 DAS, respectively) and also the CGR (12.69 g m^{-2} $days^{-1}$) due to seed inoculation as compared to no

inoculation (Table 1). Among the different fertilizer treatments the highest dry matter accumulation of 51.05 and 386.44 gm^{-2} at 30 and 60 DAS, respectively was observed (Table 1) with the treatment B_1, *i.e.* application of recommend dose of NPK, followed by that in the treatment B_2 *i.e.* application of 75 per cent recommended dose of N and P_2O_5 + 100% K_2O. Highest CGR (13.04 gm^{-2} day^{-1}) was observed (Table 1) with the treatment B_1, *i.e.* application of recommend dose of NPK, followed by that in the treatment B_2 *i.e.* application of 75 per cent recommended dose of N, P_2O_5 and 100 per cent K_2O. The lowest (37.45gm^{-2}) dry matter accumulation was recorded in the control plot where no fertilizer was applied. The interaction effects (Table 2) between two factors were different significantly at all the growth stages. The maximum dry matter accumulation was 51.18 gm^{-2} at 30 DAS with treatment A_1 x B_1 *i.e.* seed inoculation and application of recommended dose of NPK which was at par with A_1 x B_2 *i.e.* seed inoculation and application of 75 per cent recommended dose of N,

Table 1: Effect of *Rhizobium* and phosphate solubilizing bacteria (main plot) with inorganic fertilizers (sub-plot) on growth parameters of greengram (mean data of two years)

Treatments	Plant height (cm)		Dry matter accumulation (g m^{-2})		CGRg m^{-2} day^{-1}	No. of nodules $plant^{-1}$			Dry weight of nodules (g $plant^{-1}$)		
Biofertilizers	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS-60 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
A_0	18.12	46.49	40.67	366.41	12.46	27.62	44.43	27.62	0.023	0.047	0.0342
A_1	21.24	48.34	46.04	383.19	12.69	31.50	47.66	31.46	0.028	0.059	0.0413
C.D. (P=0.05)	0.3611	1.1325	2.7021	1.7745	0.4272	0.3438	1.1677	0.7037	0.0003	0.0003	0.0011
Inorganic fertilizers											
B_0	15.89	41.32	37.45	358.76	11.99	24.85	41.85	24.85	0.0205	0.040	0.029
B_1	23.96	52.55	51.05	386.44	13.04	35.46	51.63	35.37	0.0335	0.065	0.045
B_2	21.34	48.93	45.20	385.43	12.88	31.30	47.96	31.30	0.0285	0.055	0.041
B_3	17.52	46.87	39.75	368.58	12.40	26.65	42.75	26.65	0.0225	0.052	0.035
C.D. (P=0.05)	0.210	1.9656	1.7572	5.5531	0.4487	0.8031	0.8682	0.5072	0.0008	0.0008	0.0014

Treatments details: Main plots- A_0: no seed inoculation and A_1: seed co-inoculation with PSB and *Rhizobium* (M-10) strains sub plots- B_0: untreated control, B_1: application of NPK (recommended dose), B_2: 75% recommended dose of N and P_2O_5 + 100% K_2O, B_3: 50% recommended dose of N and P_2O_5 + 100% K_2O DAS= Days after sowing

Table 2:Interaction effect of *Rhizobium* and phosphate solubilizing bacteria (main plot) with a inorganic fertilizers (sub-plot) on growth parameters of greengram (mean data of two years)

Interaction (A x B)	Plant height (cm)		Dry matter accumulation (g m^{-2})		CGRg m^{-2} day^{-1}	No. of nodules $plant^{-1}$			Dry weight of nodules (g $plant^{-1}$)		
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS-60 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
A_0B_0	14.16	40.06	34.58	352.00	11.94	23.90	40.84	23.90	0.019	0.033	0.027
A_0B_1	23.55	52.14	50.93	381.70	12.95	35.50	51.51	35.50	0.034	0.062	0.045
A_0B_2	18.15	45.31	39.28	371.16	12.77	26.10	43.34	26.10	0.022	0.046	0.034
A_0B_3	16.63	48.47	37.89	360.81	12.19	25.00	42.06	25.00	0.020	0.047	0.031
A_1B_0	17.62	42.58	40.32	365.53	12.04	25.80	42.87	25.80	0.022	0.048	0.031
A_1B_1	24.38	52.96	51.18	391.18	13.13	35.42	51.75	35.24	0.033	0.068	0.046
A_1B_2	24.54	52.56	51.12	399.71	12.98	36.50	52.59	36.50	0.035	0.064	0.048
A_1B_3	18.42	45.28	41.62	376.35	12.60	28.30	43.45	28.30	0.025	0.058	0.040
S.E.± (A x B)	0.1246	0.1311	1.0431	3.2954	0.0355	0.4767	0.5152	0.3010	0.0004	0.0005	0.0005
C.D. (P=0.05)	0.3636	0.3826	3.04	9.61	0.1036	1.3912	1.5036	0.8784	0.0011	0.0014	0.0014
S.E.± (B x A)	0.1042	0.1023	0.9342	3.0027	0.0527	0.4228	0.4849	0.2763	0.0005	0.00048	0.00046
C.D. (P=0.05)	0.3041	0.2986	2.7264	8.7554	0.1538	1.2339	1.4151	0.8063	0.0014	0.0014	0.0013

Treatments details:Main plots- A_0: no seed inoculation and A_1: seed co-inoculation with PSB and *Rhizobium* (M-10) strains. Sub plots-B_0: untreated control, B_1: application of NPK (recommended dose), B_2: 75% recommended dose of N and P_2O_5 + 100% K_2O, B_3: 50% recommended dose of N and P_2O_5 + 100% K_2O DAS= Days after sowing

P_2O_5 and 100 per cent K_2O.

The magnitude of nitrogen fixation by pulse crop is determined by nodule formation. The number of nodules plant^{-1} was observed at three different stages of growth at 30, 45 and 60 DAS. There were significant differences in nodule count due to different applied fertilizers to mung bean (Table 1). The highest number of nodules per plant (31.50, 47.66 and 31.46) was found at 30, 45 and 60 DAS, respectively in the plots treated with co inoculation of seed. The lowest number of nodules per plant was recorded under the treatment A_0, *i.e.* no seed inoculation. Among the different fertilizer treatments the highest number of nodules plant^{-1} was observed 35.46, 51.63 and 35.37 at 30, 45 and 60 DAS, respectively (Table 1) in the crops grown with basal application of recommended dose of NPK followed by treatment B_2 *i.e.* application of 75 per cent recommended dose of N, P_2O_5 and 100 per cent K_2O. The lowest nodules number plant^{-1} was recorded in untreated control.Maximum number of nodule plant^{-1} of 35.42, 51.75 and 35.24 at 30, 45 and 60 DAS, respectively were recorded in the plants grown with the treatment combination seed co-inoculation along with basal application of NPK (recommended dose) which was at par with the treatment combination of seed co-inoculation and 75 per cent recommended dose of N, P_2O_5 and 100 per cent K_2O plot. In general, number of nodules plant^{-1} increased upto first 45 DAS and, thereafter, started decreasing, irrespective of all the treatments. This is mainly due to the reduction in nutrient demand of the crop as it steps forward towards maturity.

Dry weight of nodules indicates growth of the nodule. Seed inoculation also showed the significantly the highest nodule dry weight per plant (0.0028, 0.059 and 0.0413 gm plant^{-1} at 30, 45, and 60 DAS, respectively), while the corresponding lowest were (0.023, 0.047 and 0.034 g plant^{-1}) in no seed inoculation. Among the different fertilizer treatment highest nodule dry weight plant^{-1} (0.0335, 0.065 and 0.045 g plant^{-1} at 30, 45 and 60 DAS, respectively) were recorded in recommended dose of NPK and closely followed by treatment B_2 *i.e.* application of 75 per cent recommended dose of N and P_2O_5 +100% K_2O (Table 1). The nodule dry weight plant^{-1} was lowest at all the growth stages with respect to the treatment with no fertilizer application (B_0). Significant interaction effect was found at all dates of observation (Table 2), however, the best interaction effect was found with both the treatment combination A_1B_1 *i.e.* seed inoculation with and application of recommended dose of NPK as basal (0.035, 0.064 and 0.048 g plant^{-1}) and treatment combination A_1B_2 *i.e.* seed inoculation and application of 75 per cent recommended dose of N and P_2O_5 + 100% K_2O. The lowest (0.019, 0.033 and 0.027g plant^{-1}) interaction effect was found with the treatment combination A_0B_0 *i.e.* no seed inoculation and no fertilizer application. It was observed in (Table 2) that nodule dry wt. increased upto 45 DAS and then it was declined due to reduction in nodule number due to shortage of carbohydrate as the photosynthates are mostly diverted towards seed.

Yield attributes and yield :

Number of pods plant^{-1} and number of seed pod^{-1} the most important yield attributes of pulses and were recorded at the time of harvesting. From the result it was found that the highest number of pods plant^{-1} and seed pod^{-1} was recorded (26.07 and 11.42) in the seed

Table 3: Effect of *Rhizobium* and phosphate solubilizing bacteria (main plot) with inorganic fertilizers (sub-plot) on yield attributes of greengram (mean data of two years)

Treatments	Pods plant^{-1}	Seeds pod^{-1}	Seed yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Harvest index (%)
Biofertilizers					
A_0	23.695	10.9925	718.25	2484.25	22.18
A_1	26.077	11.4225	799.25	2457.25	22.49
C.D. (P=0.05)	1.6215	0.3069	16.65	24.6192	0.5016
Inorganic fertilizers					
B_0	20.275	9.735	632.5	2309	21.22
B_1	28.795	12.23	869.5	2638	23.23
B_2	28.17	12.255	833	2577.5	22.85
B_3	22.305	10.61	700	2358.5	22.04
C.D. (P=0.05)	1.4405	0.3744	16.16	25.1793	0.5799

Treatments details: Main plots-A_0: no seed inoculation and A_1:seed co-inoculation with PSB and *Rhizobium* (M-10) strains. Sub plots-B_0: untreated control, B_1: application of NPK (recommended dose), B_2: 75% recommended dose of N and P_2O_5 + 100% K_2O, B_3: 50% recommended dose of N and P_2O_5 + 100% K_2O DAS= Days after sowing

inoculation (Table 3). Among the different fertilizer treatments significantly the highest (28.79) number of pods plant^{-1} and 12.23 seed pod^{-1} were recorded in recommended dose of NPK followed by treatment B_2 *i.e.* application of 75 per cent recommended dose of N and P_2O_5 + 100% K_2O (Table 3). The maximum values of pods plant^{-1} (30.10) were observed both under the treatment A_1B_1 *i.e.* seed inoculation with application of recommended dose of NPK as basal and treatment combination A_1B_2 *i.e.* seed inoculation and application of 75 per cent recommended dose of N and P_2O_5 + 100% K_2O (30.85). The maximum values of seed pod^{-1} were observed both under the treatment A_1B_1 *i.e.* seed inoculation with application of recommended dose of NPK as basal (12.27) and treatment combination A_1B_2 *i.e.* seed inoculation and application of 75 per cent recommended dose of N and P_2O_5 + 100% K_2O (12.81). Singh *et al.* (2010) found similar results with lentil. Corresponding the lowest number of pods plant^{-1} (19.86) and seed pod^{-1} (9.52) were observed in the control. Significant interaction effect was found due to different treatment combinations (Table 4). It was found that seed co-inoculation with *Rhizobium* and phosphate solubilizing bacteria (*Bacillus polymyxa*) increased the pods plant^{-1} as well as seed pod^{-1} in mung bean. The increase in grain yield with nitrogen and phosphorus application might be due to higher number of pods plant^{-1} and grains pod^{-1} as grain yield is known to have positive association with these characters (Lavanya and Toms, 2009).

From the result it was revealed that a significant increase in seed yield and straw yield of mung bean was found with seed co-inoculation than un-inoculated treatment (Table 3). The highest seed yield (799 kg ha^{-1}) and straw yield (2457 kg ha^{-1}) addressed with seed co-inoculation. This finding corroborated with the result of Yousuf *et al.* (1989). Application of recommended dose of NPK increased seed yield and straw yield significantly (Table 3) over control. Recommended dose of NPK produced the highest seed yield (869 kg ha^{-1}). Maximum straw yield (2638 kg ha^{-1}) was recorded against the treatment with recommended dose of NPK (B_1) and lowest (2309 kg ha^{-1}) against treatment with no fertilizer application B_0. Among the different interaction combination the maximum seed yield (939 kg ha^{-1}) and straw yield (2648 kg ha^{-1}) was observed with A_1B_1 *i.e.* seed inoculation and along with recommended dose of NPK) which is at par with 927 kg ha^{-1} and 2662 kg ha^{-1} seed yield and straw yield, respectively, in the treatment combination A_1B_2 *i.e.* seed inoculation and application of 75 per cent recommended dose of N and P_2O_5 + 100% K_2O. The lowest seed yield (649 kg ha^{-1}) and stalk yield (2310 kg ha^{-1}) was obtained with the treatment combination A_0B_0 *i.e.* untreated control plot. Similar findings also reported by Choudhary *et al.* (2011). Significant difference was found in harvest index in different treatment combination (Table 3). The highest harvest index (22.49%) was observed in seed co-innoculation. Recommended dose of NPK showed the highest harvest index (23.23%) of mung bean. Among all the treatments significantly the highest harvest index (23.21%) was obtained in the treatment combination A_1B_2 *i.e.* seed inoculation and application of 75 per cent recommended dose of N and P_2O_5+100% K_2O (Table 4).

Table 4: Interaction effect of *Rhizobium* and phosphate solubilizing bacteria (main plot) with inorganic fertilizers (sub-plot) on yield attributes of greengram (mean data of two years)

Treatments	Pods plant^{-1}	Seeds pod^{-1}	Seed yield (kg ha^{-1})	Straw yield(kg ha^{-1})	Harvest index(%)
A_0B_0	19.86	9.52	649	2310	20.65
A_0B_1	27.49	12.19	800	2628	23.25
A_0B_2	25.49	11.70	739	2563	22.43
A_0B_3	21.94	10.56	685	2436	22.40
A_1B_0	20.69	9.95	616	2308	21.80
A_1B_1	30.10	12.27	939	2648	23.21
A_1B_2	30.85	12.81	927	2662	23.28
A_1B_3	22.67	10.66	715	2281	21.69
S.E.± (A x B)	0.8549	0.2230	7.031	14.9432	0.3441
C.D. (P=0.05)	2.4950	0.6508	20.51	43.6116	1.004
S.E.± (B x A)	0.7310	0.7202	6.272	13.622	0.3062
C.D. (P=0.05)	2.133	2.101	18.30	37.75	0.8936

Treatments details: Main plots- A_0: no seed inoculation and A_1: seed co-inoculation with PSB and *Rhizobium* (M-10) strains.
Sub plots- B_0: untreated control, B_1: application of NPK (recommended dose), B_2: 75% recommended dose of N and P_2O_5 + 100% K_2O, B_3: 50% recommended dose of N and P_2O_5 + 100 % K_2O DAS= Days after sowing

Conclusion :

From the above experiment it was observed that inoculation with *Rhizobium* and phosphate solubilizing bacteria (PSB) along with 75 per cent RDF *i.e.* treatment combination (A_1B_2) was at par with treatment A_1B_1 *i.e.* 100 per cent RDF with respect to all the growth parameters and yield attributing characters of mungbean. So, it can be concluded that both 25 per cent nitrogenous and phosphatic fertilizer of the recommended dose can be substituted by seed co- inoculation with phosphate solubilizing bacteria and *Rhizobia* without affecting the yield compared to 100 per cent RDF.

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